
USE OF ZPT AND PLANTING MEDIA ON THE GROWTH OF MYCELIUM
OYSTER MUSHROOM (*Pleurotus ostreatus*)

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ABSTRACT

White Oyster mushroom (*Pleurotus ostreatus*) is an alternative food for the society because of its high nutrients content. This study uses of ZPT and media to see the grow of the mycelium white oyster mushroom. Media used are the boiling water extract of nuts (green beans, soy bean, peanuts, beans of string bean, and beans of nut snaps) and ZPT (Naphthalene Acetic Acid and Kinetin). Anova was used to analyze the data at a significant level of $\alpha = 0.05$. The results shows that: (1) There's a significant effect of ZPT and Non-ZPT on the mycelium growth with $p=0.000$, Duncan Multiple Range Test (DMRT) shows that Kinetin is highest contribution to the significancy of Anova; (2) planting media used in the study shows a significant differences on the mycelium growth with $p=0039$, Duncan Multiple Range Test shows that beans of long bean and soy bean are the highest contribution to the significancy of Anova; (3) time/days of observation done in the study shows a significant differences on the mycelium growth with $p=0000$, Duncan Multiple Range Test shows that T6 or day 14 of observation shows the highest contribution to the significancy of Anova; (4) the interaction of ZPT, Non-ZPT and planting media show a significant differences on the mycelium growth $p=0000$; (5) the interaction of ZPT, Non-ZPT and time show a significant differences on the mycelium growth with $p=0000$; (6) the interaction of media and planting time show a significant differences on the mycelium growth with $p=0000$; and (7) the interaction of ZPT, Non-ZPT and planting media and time do not affect significantly the mycelium growth with $p=0053$.

Key Word: Oyster mushroom (*Pleurotus ostreatus*), ZPT, Tissue Culture, Growth media.

INTRODUCTION

Mushrooms are food alternative often consumed in Indonesia, it is easy to cultivate and have a high business prospect. The kind of mushroom frequently consume among other mushrooms is the white oyster mushroom (*pleurotus ostreatus*), ear mushroom (*auricula polytricha*), lingzhi (*ganoderma lucidum*), and abalone (*pleurotus abalonus* or *pleurotus cystidiosus*), and Shiitake (Jamin, 2010 and Suparti et.al., 2016). Today, the role of edible mushroom to have a prospect high trade, expensive and exported make edible mushroom the right choice to be cultivated (Abbas et.al. , 2011).

In agriculture, white oyster mushroom is a known good business since this mushroom adapt well to many environment and high levels of productivity. Besides of the nature of adaptation and high levels of productivity, white oyster mushroom also has a high nutritional value as well as consist of protein at 27%, twice compare to asparagus, cabbage, and potatoes, four times to carrot and tomato, and 6 times to orange (authority, 2012).

White oyster mushroom has its medicinal factor, its use to cure liver disease, diabetes, and anemia. It's also good as antivirus, antibacterial, antitumor, and anticancer. It also reduces body weight and help in digestion (Wibawa, 2012).

Mushroom growth is influenced by several factors, one of which is ZPT (Growth Regulating Substances). According to Handoko (2011) Auxin increases the grow of the stem, fruit development, and root branching. While cytokinin play a role in the process of growth and differentiation of roots, helps cell division, growth in General, help germination and slow down aging. ZPT acted as organogenesis controller and "morphogenesis" in the process of formation and development of shoots, roots, and the establishment of calus. In the balance combination of ZPT and basic media grow it

increases the activity in the cell division process "morphogenesis" as well as organogenesis (Lestari, 2011).

The media use for planting should have superior nutritional value, according to Kuswardhani (2016) in addition to the nutrient content of the legume pertained to superior, beans also have some content of active substances, one of which is a phytochemical compounds. Phytochemicals are conceived by nuts are not required in the metabolic processes of the body but the presence of these phytochemicals have effects that are very good for health because it can prevent the disease in the body.

Based to the above background, this research is done on how to accelerate the growth of white oyster mushroom faster through tissue culture, and by mixing the growth media and ZPT, NAA and Kinetin..

RESEARCH METHODS

This is an experimental research design using laboratory tools such as, laminar air flow cabinet, autoclave, digital weighing scale, magnetic stirrer, electric stove, bottles, petridish, measuring glass, knife, scissors, test tubes, bunsen burner, plastic, rubber, hotplate, incubator, spatula, and bottles.

Growth materials used are, Growth media such as green beans, soybean, peanuts, long beans, and plant growth regulating substances, NAA and kinetin.

The subject used in the study is white oyster mushrooms (*pleurotus ostreatus*) explant. Mushroom is in good condition, thick mycelium and uncontaminated by fungi and bacteria. Explant is part of a plant which is used as a primordia for plant propagation.

The object of this research is the growth regulating substance, they are NAA and Kinetin. They are mixed with growth media.

Concept and Operation Variables

The independent variables are ZPT (NAA and Kinetin and growth media, and the dependent variable is the growth, in length, of the mycelium.

ZPT used is 0.5 ml per 250 ml solution media. The explant used is white oyster mushroom (*pleurotus ostreatus*), in this research was termed as f_0 .

Research Procedure

After the sterilization of the tools needed, the medium used in this research was made. Medium used in in this research was long beans, beans, peanuts, green beans, and soybean. Part of nuts used is exactly the nut, not the skin or the concluding part of the nuts.

In the process of making the media, the first stage is weighing the nuts, each nut weighed 200 gr, and then the nuts was soaked in the 250ml distilled water or until covers all the nuts. The soaking time is 1 night. Then the nuts were dried, and after that was soaked in 250ml alcohol or until cover whole beans the location for 3 to 5 seconds, and then washed using distilled water.

After the soaking processes, the nuts were boiled in 250ml water for 15 minutes. The stewed water was then drained and collected, and then was mixed with 10 grams of white sugar and added distilled water until the volume is about 500 ml and the pH is 6.5 – 7 (ideal for white oyster mushroom growth). After all of material were mixed, the media was then divided into 2 parts each were 250 ml. Then 0,5 ml of NAA and 0,5 ml of Kinetin were added into each part of the media. Those media were then poured into 5 bottles, each contain 20 ml, the bottles were then sterilized for 60 minutes in an autoclave. After every bottle that contains the media has ben sterile, the bottles saved to in storage room for 1 night, the white oyster mushroom was planted.

The explant (f_0) used in this research was obtained from the tissue culture laboratory (BALITSA). The white Oyster mushroom was grown in a plant media which have provided, with the position of the explant is on the surface of media. In the process inoculation all the equipment used has to be really sterile and that the explant is uncontaminated. All of this were done in a sterilized room and at the room temperature. Inoculation was done in a laminar air flow cabinet that also has been sterilized.

Observation growth mycelium

The incubation of the explant were done in petri dish for 5-10 days after inoculation. The growth of the mycelium, in length, were observed and measured every other day for 14 days. The data collection process in this research was as follows:

1. T0: inoculation
2. T1: the data on the 3rd day;
3. T2: the data on the 5th day;
4. T3: the data on the 7th day;
5. T4: the data on the 10th day;
6. T5: the data on the 12th day; and
7. T6: the data on the 14th day.

Data analysis

Data obtained was the processed and analyzed statistically, using graphic diagrams and average measurement of the change in growth of the mycelium of white oyster mushroom (*pleurotus ostreatus*). A chart of change growth is used for described and explains the growth rate of any treatment given. Data analysis used in this research is statistical tests using analysis of variance (ANOVA) and the simple regression linear. Statistical tests is used to see the difference in the average measurements of growth between media using ZPT, NAA and Kinetin, and media without ZPT in the six times data collection in measuring the mycelium growth as of the mycelium growth. If the result of the of ANOVA show a significant difference, the Duncan's multiple range test (DMRT) was used.

RESULTS AND DISCUSSION

Below is the descriptive analysis of data in average of ZPT and Non-ZPT, Plant media, and time.

1. hormone

The table below show the average mycelium growth between ZPT and Non-ZPT of the white oyster mushroom (*pleurotus ostreatus*).

Table 1. Average Growth (cm) of White Oyster Mushroom (*pleurotus ostreatus*) Mycelium Based on ZPT and Non-ZPT

Hormone	Mean	SE	95% interval confidence	
			Lower limit	Upper limit
Non-ZPT	4.311	.056	4.201	4.421
NAA	4.965	.056	4.855	5.075
Kinetin	5.038	.056	4.927	5.148

From the table above we can see that kinetin has the highest growth rate 5.038 and control the lowest 4.311.

2. Media

Below is the descriptive analysis of the mycelium growth of white oyster mushroom (*pleurotus ostreatus*) based on the plant media used.

Table 2. Average Growth (cm) of White Oyster Mushroom (*pleurotus ostreatus*) Mycelium Based on Plant Media

Media	Mean	SE	95% Interval confidence	
			Lower limit	Upper limit
Long beans	4.881	.072	4.738	5.023

Beans	4.754	.072	4.612	4.896
Peanut	4.593	.072	4.451	4.735
Green beans	4.761	.072	4.619	4.903
Soya beans	4.867	.072	4.725	5.009

From the table above we can see that long beans is the highest 4.881 and peanuts the lowest 4.593.

3. Time

Below is the descriptive analysis of the mycelium growth of white oyster mushroom (*pleurotus ostreatus*) based on the time of measurement.

Table 3. Average Growth (cm) of White Oyster Mushroom (*pleurotus ostreatus*) Mycelium Based on Time of Measurement.

Time	Mean	SE	95% Interval Confidence	
			Lower limit	Upper limit
T1	2.273	.079	2.118	2.429
T2	3.508	.079	3.353	3.664
T3	4.745	.079	4.589	4.901
T4	5.402	.079	5.246	5.557
T5	5.963	.079	5.808	6.119
T6	6.735	.079	6.579	6.891

From the table above the result shows that T6 or 14th day of harvest is the highest 6.891 and T1 or 1st day of harvest is the lowest 2.273.

Homogeneity Test

The table below show results test of homogeneity from the data long mycelium oyster mushroom white.

Table 4. Data Homogeneity Test on the Growth (cm) of White Oyster Mushroom (*pleurotus ostreatus*) Mycelium.

F	df1	df2	Sig.
7.840	89	270	.000

From the table of homogeneity test above, it shows that the data obtained is homogeneous, seen from the result of significant where $p = 0.000 (< \alpha = 0.05)$. Because of data collected is homogeneous hence the analysis then be continued by using ANOVA factorial 3x5x6, where there are 3 main the type of treatment ZPT (NAA, Kinetin) and Non-ZPT, 5 kinds of media, and six times the time data collection.

Data Analysis

table below show results statistical tests from the data long mycelium oyster mushroom white.

Table 5. Data Analysis (ANOVA) of Mycelium Growth of White Oyster Mushroom (*pleurotus ostreatus*)

Source	SS	DF	MS	F	Sig.
Corrected Model	1098.565	89	12.343	32.901	.000
Mencegat	8194.860	1	8194.860	21843.253	.000
ZPT	38.449	2	19.225	51.243	.000
Media	3.830	4	.958	2.552	.039
Time	810.602	5	62.120	432.129	.000

ZPT*Media	184.309	8	23.039	61.409	.000
ZPT*Time	14.712	10	1.471	3.921	.000
Media*Time	25.187	20	1.259	3.357	.000
ZPT*Media*Time	1.475	40	.537	1.431	.053
Error	101.295	270	.375		
Total	9394.720	360			
Corrected Total	1199.860	359			

1. ZPT and Non-ZPT

The table above shows significance difference in the treatment ZPT where $p = 0.000$ ($< \alpha = 0.05$), thus the hypothesis that said, “There is no significant effect of ZPT and Non-ZPT on the growth of mycelium of white oyster mushroom (*pleurotus ostreatus*)”, is rejected. This means that growth regulating substance significantly affect the mycelium growth of white oyster mushroom. Since the result is significant, Duncan’s Multiple Range Test (DMRT) is use to determine which ZPT or Non-ZPT that gives the significant result.

Table 6. Duncan’s Multiple Range Test on Mycelium Growth Based on ZPT and Non-ZPT.

ZPT	N	Subset		Duncan’s notation
		1	2	
Non-ZPT	120	4.3108		a
NAA	120		4.9650	b
Kinetin	120		5.0375	b
Sig.		1.000	.360	

From the table above we found that NAA and Kinetin give the greatest effect to the significance in rejecting the hypothesis of the study.

2. Media

Table 5 above shows that plant media effect significantly the mycelium grow with $p = 0.039$ ($< \alpha = 0.05$) this shows that the hypothesis that said “There is no significant effect of various plant media on the growth of mycelium of white oyster mushroom (*pleurotus ostreatus*)”, is rejected. This means that plant media significantly affect the mycelium growth of white oyster mushroom. Since the result is significant, Duncan’s Multiple Range Test (DMRT) is then use to determine which plant media that gives the significant result.

Table 7. Duncan’s Multiple Range Test on Mycelium Growth based on the Kinds of Plant Media.

Media	N	Subset		Duncan’s notation
		1	2	
Peanut	72	4.5931		a
Beans	72	4.7542	4.7542	ab
Green beans	72	4.7611	4.7611	ab
Soya beans	72		4.8667	b
Long beans	72		4.8806	b
Sig.		.121	.265	

The table above shows that long beans and soya beans as plant media gives the greatest impact on the growth of the mycelium.

3. Time

Table 5 above shows that the time of measurements effect significantly the mycelium grow with $p = 0.000$ ($< \alpha = 0.05$) this shows that the hypothesis that said “There is no significant effect of time of measurement on the growth of mycelium of white oyster mushroom (*pleurotus ostreatus*)”, is rejected. This means that time of measurement significantly affect the mycelium growth of white

oyster mushroom. Since the result is significant, Duncan's Multiple Range Test (DMRT) is then used to determine which time of measurement that gives the significant result.

Table 8. Duncan's Multiple Range Test on Mycelium Growth Based on the Time of Measurement.

Time	N	Subset						Duncan's Notification
		1	2	3	4	5	6	
T1	60	2.27						a
T2	60		3.51					b
T3	60			4.75				c
T4	60				5.40			d
T5	60					5.96		e
T6	60						6.74	f
Sig.		1.00	1.00	1.00	1.00	1.00	1.00	

The table above shows that T6 or 14th day of measurement gives a greatest impact to the growth of the mycelium.

4. ZPT, Non-ZPT and Plant Media

Table 5 above shows that the interaction between growth regulating substance and plant media effect significantly the mycelium grow with $p = 0.000 (< \alpha = 0.05)$ this shows that the hypothesis that said "There is no significant effect of the interaction between ZPT, Non-ZPT and plant media on the growth of mycelium of white oyster mushroom (*pleurotus ostreatus*)", is rejected. This means that the interaction between ZPT, Non-ZPT, and plant media significantly affect the mycelium growth of white oyster mushroom.

5. ZPT, Non-ZPT and Time of Measurement

Table 5 above shows that the interaction between growth regulating substance and time of measurement effect significantly the mycelium grow with $p = 0.000 (< \alpha = 0.05)$ this shows that the hypothesis that said "There is no significant effect of the interaction between ZPT, Non-ZPT and time of measurement on the growth of mycelium of white oyster mushroom (*pleurotus ostreatus*)", is rejected. This means that the interaction between ZPT, Non-ZPT, and time of measurement significantly affect the mycelium growth of white oyster mushroom.

6. Plant Media and Time of Measurement

Table 5 above shows that the interaction between growth regulating substance and plant media effect significantly the mycelium grow with $p = 0.000 (< \alpha = 0.05)$ this shows that the hypothesis that said "There is no significant effect of the interaction between ZPT, Non-ZPT and time of measurement on the growth of mycelium of white oyster mushroom (*pleurotus ostreatus*)", is rejected. This means that the interaction between ZPT, Non-ZPT, and time of measurement significantly affect the mycelium growth of white oyster mushroom.

7. ZPT, Non-ZPT, Plant Media and Time of Measurement

Table 5 above shows that the interaction between growth regulating substance and plant media effect significantly the mycelium grow with $p = 0.000 (< \alpha = 0.05)$ this shows that the hypothesis that said "There is no significant effect of the interaction between ZPT, Non-ZPT, plant media, and time of measurement on the growth of mycelium of white oyster mushroom (*pleurotus ostreatus*)", is rejected. This means that the interaction between ZPT, Non-ZPT, plant media, and time of measurement significantly affect the mycelium growth of white oyster mushroom.

CONCLUSION

This research which have been done to study on the effect of ZPT, Non-ZPT, plant media, time of measurement, and the interaction between the variables concluded that:

1. Growth regulating substance, ZPT and Non-ZPT, effect the growth mycelium of white oyster mushroom with $p = 0.000$ ($< \alpha = 0.05$). Duncan's Multiple Range Test (DMRT) duncan shows that NAA and kinetin give the greatest significance in the rejection of the hypothesis
2. Media nuts influenced the growth mycelium oyster mushroom white with value $p = 0.039$ ($< \alpha = 0.05$). The distance multiple duncan shows that variable long beans , green beans , and peanuts beans give the highest significance in the rejection of the hypothesis.
3. Affecting time growth mycelium oyster mushroom white with value $p = 0.000$ ($< \alpha = 0.05$). The distance multiple duncan shows that variable time t6 (the data on day 14th) give the highest significance in the rejection of the hypothesis.
4. Interaction zpt, non-zpt in a media influenced the growth mycelium oyster mushroom white with value $p = 0.000$ ($< \alpha = 0.05$).
5. Interaction zpt, non-zpt in the influenced the growth mycelium oyster mushroom white with value $p = 0.000$ ($< \alpha = 0.05$).
6. The interaction of nuts on media affecting time underground growth of the mycelium of oyster mushrooms white with the value of $p = 0.000$ ($< \alpha = 0.05$).
7. Interaction zpt , non-zpt in a media nuts and time did not affect the growth of the mycelium of a fungus oysters white with the value of $p = 0.053$ ($\alpha > = 0.05$).

SUGGESTIONS

to make during the process further research work better and objectives the research you want reached hence writers suggest as follows:

1. Needs to be done advanced research using same media against the type other fungi by using the method tissue culture.
2. Needs to be done advanced research on the use of zpt other help grow mycelium oyster mushroom .
3. Attention should be given to sterility room to does not occur contamination .

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